Neuron, Vol. 46, 373–376, May 5, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.neuron.2005.04.014

Cortical Neuron Specification: It Has Its Time and Place

Minireview

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Cortical neurogenesis is a highly stereotyped process in which progenitor cells generate neurons destined for specific cortical layers depending on the timing of cell cycle exit. Previous work has shown that during corticogenesis, progenitors become progressively restricted in their developmental potential. Recent work has uncovered some of the intrinsic mechanisms that underlie this fate restriction. In addition to timing, new studies suggest that the location of cell cycle exit in the cortical germinal zone may also contribute to cortical neuron specification.

The cerebral cortex is a highly ordered brain structure with neurons organized into distinct layers each displaying unique afferent and efferent connections. Cortical neurons can be broadly divided into two classes: interneurons and projection neurons. The interneurons are a varied subgroup of cells, which occupy many different cortical layers and largely utilize GABA as a neurotransmitter. In rodents, the vast majority of cortical interneurons derive from progenitors located in the ventral telencephalon and migrate tangentially to populate the forming cerebral cortex (reviewed in Marin and Rubenstein, 2001). In humans, however, the cortical interneurons also derive, at least in part, from progenitors located in the cortical germinal zone (Letinic et al., 2002).

Unlike cortical interneurons, the cortical projection neurons utilize glutamate as a neurotransmitter and derive from progenitors located exclusively in the dorsal telencephalon (also known as the pallium) (Gorski et al., 2002). These neurons migrate radially from the germinal zones into the cortical plate. The layer that these neurons will ultimately occupy depends on the time at which they become postmitotic, such that early born neurons reside in the deep layers (i.e., V and VI), whereas the later-generated neurons occupy layers II–IV (reviewed in Rice and Curran, 2001). Within each of these layers, the projection neurons can exhibit axonal connections with distinct cortical areas, subcortical regions (including the spinal cord), or the opposite hemisphere of the cortex via the corpus callosum.

Cortical neurogenesis occurs over a protracted period as compared to more caudal regions of the brain. Although the first cortical neurons (preplate) are born already at midgestation stages in the mouse, the last cortical neurons (layers II/III) emerge around birth. This is in contrast to the spinal cord where neurogenesis is complete well before birth. Previous transplantation studies have shown that cortical progenitors become progressively restricted in their developmental potential during corticogenesis, such that progenitors at early stages of cortical neurogenesis are multipotent and can generate projection neurons of most layers, whereas the later-stage progenitors are restricted to forming only the upper layers (McConnell and Kaznowski, 1991; Frantz and McConnell, 1996; Desai and McConnell, 2000). These studies suggest that cortical progenitors become intrinsically restricted in their developmental potential over time. A recent study (Mizutani and Saito, 2005) suggests that the environment of the cortical germinal zone may play a role in this progressive fate restriction. In this study, some early cortical progenitors were blocked from undergoing neurogenesis by expressing a constitutively active (ca)Notch receptor. By using a cre-based recombination approach, the ca-Notch was recombined out at later stages of corticogenesis, and the double-transfected cells were able to undergo neurogenesis. Although these cells were maintained in a progenitor state from early neurogenic periods, they nevertheless produced upper layer neurons similar to their untransfected late-stage counterparts. These findings suggest that cortical progenitors do not need to undergo neurogenesis for fate restriction to occur. Rather, changes in extrinsic signals within the progenitor environment during cortical development may contribute to the progressive restriction of developmental potential.

A number of transcription factors have been implicated in the generation of cortical neurons. These factors have been suggested to promote the acquisition of cortical neuron phenotypes as well as to repress genetic programs that would regulate noncortical neuronal differentiation such as those operating in the ventral telencephalon. The paired-homeodomain transcription factor Pax6 and the bHLH factors Neurogenin (Ngn)1 and 2 have been implicated in this latter role (Fode et al., 2000; Stoykova et al., 2000; Toresson et al., 2000; Yun et al., 2001; Schuurmans et al., 2004). In the absence of these factors, cortical progenitors are misspecified, expressing genes typical of ventral telencephalic progenitors. The result seems to be a respecification toward ventral neuronal fates. Indeed, the cells generated appear molecularly more akin to the GABAergic interneurons produced in the ganglionic eminences (Fode et al., 2000; Yun et al., 2001; Schuurmans et al., 2004).

The first neurons generated in the developing cerebral cortex form the preplate, which splits and gives rise to the marginal zone (layer I) and the subplate (reviewed in Rice and Curran, 2001). From this stage on, all cortical development is inside out, i.e., the deep layers are generated first, followed by the upper layers. The cells in layer I are interneurons called Cajal-Retzius (CR) cells and have been shown to be important for the inside-out development of the subsequent cortical layers by virtue of the expression of Reelin (Rice and Curran, 2001). A recent study by Hanashima et al. (2004) showed that the telencephalon-specific transcription factor Foxg1 (previously known as Bf-1) actively re-

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presses the generation of CR cells at later stages of cortical neurogenesis. In the *Foxg1* mutants, the entire telencephalon is dramatically reduced in size (Xuan et al., 1995), indicating the general importance of this factor for the expansion of telencephalic progenitors. However, the rudimentary cortex present in these mutants contains a remarkable number of neurons that exhibit phenotypes of CR cells. Interestingly, if *Foxg1* gene expression is disrupted when deep layer cortical neurons are being produced, the progenitors appear to revert to generating CR fates. Whether subplate neurons, which are generated around the same time as the CR cells in the preplate, are equally affected in these mutants remains unclear. It is important to mention that a recent study (Takiguchi-Hayashi et al., 2004) has shown that many CR cells derive from a site adjacent to the forming neocortex, the caudomedial wall of the telencephalon. Thus, the *Foxg1* mutant phenotype could also represent an alteration in spatial patterning, with CR-producing domains expanded at the expense of neocortical domains. In any case, Foxg1 represents an intrinsic molecular determinant, which suppresses CR neuron development and thereby restricts the developmental potential of cortical progenitors (Figure 1). *Foxg1* is expressed by the vast majority of telencephalic progenitors (Tao and Lai, 1992); however, CR neurons appear to exclude its expression (Hanashima et al., 2004). This is despite the fact that at least some of the CR cells derive from *Foxg1*-expressing progenitors (Hanashima et al., 2004). Therefore, the mechanisms that work to suppress *Foxg1* expression in these earliest cortical progenitors/neurons will be important to uncover. Also, it will be interesting to determine whether Foxg1 continues to suppress CR cell fate into the latest stages of cortical neurogenesis when upper layer cortical neurons are being generated. In addition to Foxg1, the T-box transcription factor Tbr1 has also been shown to be important for preplate formation, including the correct differentiation of both CR cells and subplate neurons (Hevner et al., 2001; Figure 1).

After the preplate is formed, the cortical progenitors generate almost exclusively projection neurons (because most cortical interneurons in the rodent are generated in the ventral telencephalon). As mentioned above, the laminar fate of these projection neurons is largely dependent on the timing of cell cycle exit. The *Ngns* are expressed in cortical progenitors throughout the entire period of cortical neurogenesis and are required to repress ventral telencephalic identity (Fode et al., 2000). A recent study has shown that this requirement only exists at early stages of corticogenesis (Schuurmans et al., 2004; Figure 1). The absence of *Ngn2* or both *Ngn1* and *Ngn2* results in severe alterations of deep layer neurons, whereas increased numbers of GABAergic neurons, typical of those derived from the ventral telencephalon, are found. Remarkably, at late stages of cortical development, the *Ngn*-deficient cortical progenitors do produce upper layer neurons of an apparently normal phenotype (Schuurmans et al., 2004). Consistent with its expression in deep layer neurons, Tbr1 is also required for the correct formation of layer VI neurons, but not upper layer neurons (Hevner et al., 2001; Figure 1). Although the generation of upper layer projection neurons seems to be *Ngn* and *Tbr1* independent, this process does require the function of two other transcription factors, Pax6 and the nuclear orphan receptor Tlx (also known as tailless and NR2E1) (Tarabykin et al., 2001; Land and Monaghan, 2003; Schuurmans et al., 2004). These two factors have been shown to cooperate genetically to pattern the lateral telencephalon including the pallio-subpallial boundary (Stenman et al., 2003), and the combined absence of *Pax6* and *Tlx* leads to an almost complete loss of upper layer formation, at least at rostral levels of the cortex (Schuurmans et al., 2004). This suggests that despite the morphological similarities between the deep and upper layer cortical neurons, distinct genetic programs are required for their normal production (Figure 1). Whether this is indicative of the different roles that these cortical layers will perform or simply an evolutionary add on when vertebrate cortices became laminated is presently unclear.

In spite of their apparent requirement for late-stage corticogenesis, Pax6 and Tlx are expressed by cortical

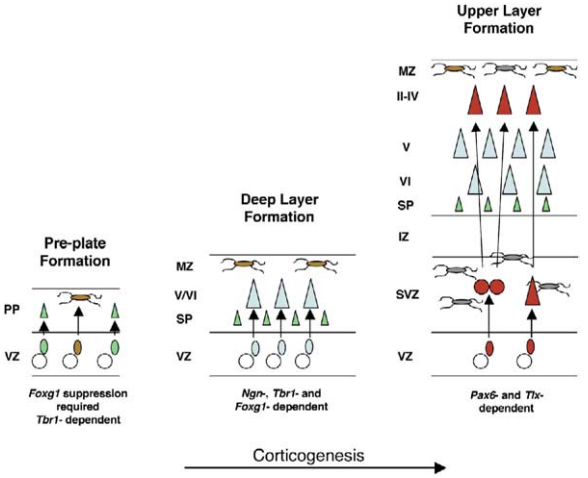

Figure 1. Schematic Showing Three Progressive Stages of Corticogeneisis: Preplate Formation, Deep Layer Formation, and Upper Layer Formation

The formation of the preplate (PP) requires the expression of *Tbr1*, whereas the generation of Cajal-Retzius (CR) neurons (brown cells) involves the suppression of *Foxg1* at the earliest stages of corticogenesis. After the PP splits to form the marginal zone (MZ) and subplate (SP, green cells), deep layer (V and VI, light blue cells) neurons are generated and require the function of both *Ngns* and *Tbr1* (at least for layer VI). At this time, *Foxg1* is also required to prevent CR neuron generation in place of the deep layer neurons. When upper layer cortical neurons (II–IV, red cells) are generated, *Ngns* and *Tbr1* appear to be dispensable, but the functions of *Pax6* and *Tlx* are absolutely required. The specific roles of these two factors are unclear; however, they are required for the correct formation of the cortical SVZ. This secondary germinal zone is likely to play an important role in specifying upper layer identities in the late-generated cells, which can be born in either the SVZ (by symmetric division of VZ-derived progenitors) or in the VZ through asymmetric division. SVZ progenitors express a number of factors distinct from the VZ that are likely to contribute to the specification of upper layer neurons. In addition, the ventrally derived GABAergic interneurons (gray cells) that populate the SVZ represent another potential source of specifying signals. Abbreviation: IZ, intermediate zone.

progenitors throughout corticogenesis. How then are they able to restrict the developmental potential of these progenitors over time? One possibility is the role they might play in forming the subventricular zone (SVZ). Telencephalic neurogenesis is characterized by the emergence of two distinct germinal zones. Although most parts of the brain contain only a proliferative ventricular zone (VZ), the telencephalon exhibits a secondary germinal zone termed the SVZ. The cortical SVZ has recently been suggested to generate many of the upper layer neurons (Tarabykin et al., 2001). In this study, a gene, *Svet1*, was shown to be expressed in the cortical SVZ and subsequently in the upper layer neurons of the cortical plate. In *Pax6* mutants, *Svet1* is lost in both the SVZ and cortical plate (Tarabykin et al., 2001). At least two more genes, *Cux2* (Nieto et al., 2004; Zimmer et al., 2004) and *Satb2* (Britanova et al., 2005), have since been described that mark the SVZ and upper layers of the cortex. The expression of *Cux2* is also altered in *Pax6* mutants (Nieto et al., 2004; Zimmer et al., 2004). It is interesting to note that widespread ventral misspecification in the *Pax6* mutant cortex (as discussed earlier) occurs at a later time point (i.e., roughly 2 gestational days later) than it does in *Ngn* mutants (Fode et al., 2000; Toresson et al., 2000; Yun et al., 2001). This delay fits well with the timing of cortical SVZ formation. Thus, Pax6 is required for the normal molecular identity of cortical SVZ progenitors. In addition to the molecular abnormalities, the *Pax6* mutant SVZ exhibits increased numbers of cycling cells (Toresson et al., 2000; Yun et al., 2001). Alterations in cortical SVZ proliferation have also been described for the *Tlx* mutants (Roy et al., 2004). Therefore, these two factors are essential for the normal formation of the cortical SVZ and thereby may regulate upper cortical layer formation.

The specific manner in which *Pax6* and *Tlx* contribute to SVZ formation will be important to uncover. A recent study suggests that *Ngn2*, a target of Pax6 (Scardigli et al., 2003), can promote the generation of basal progenitors, which may seed the cortical SVZ (Miyata et al., 2004). It's unclear, however, how that fits with the apparent *Ngn*-independent generation of upper layer cortical neurons. *Mash1* is misexpressed in *Ngn* mutant cortical progenitors (Fode et al., 2000; Schuurmans et al., 2004), and perhaps at later stages (in the presence of Pax6), it can function in a similar manner in promoting SVZ formation and upper layer fates. In fact, in embryos where *Mash1* replaced the *Ngn2* locus (i.e., knockin), only deep cortical layer development is affected, similar to that in the *Ngn* mutants (Schuurmans et al., 2004). In addition to intrinsic determinants, recent work has also shed light on the signaling pathways that contribute to SVZ formation (Viti et al., 2003). Wnt7a and 7b appear to cooperate with Sonic hedgehog and fibroblast growth factor 2 signaling to promote the maturation of progenitors from a VZ to SVZ state. These factors may thus contribute to the progressive restriction of cortical progenitors by promoting changes in their intrinsic properties.

The cellular processes involved in the formation of the cortical SVZ have only recently begun to be understood. Recent studies (Noctor et al., 2004; Haubensak et al., 2004; Miyata et al., 2004) have shown that the cells in the VZ provide progenitors to the SVZ via asymmetric division. These SVZ progenitors were shown to subsequently divide symmetrically to generate either two neurons (Figure 1) or two more progenitors. In this model, the SVZ does not maintain itself but rather relies entirely on the VZ for its progenitors. It is unclear whether this is also the case for the SVZ of the ventral telencephalon. Fate mapping studies using the *GFAP-cre* mice might argue against it (Malatesta et al., 2003). The *GFAP* promoter is restricted to radial glial cells in the VZ of the telencephalon and does not become highly expressed until midneurogenesis. Although most cortical neurons are recombined (i.e., fate mapped) with this cre driver, very few cells in the ventral telencephalon are. This suggests that the ventral telencephalic SVZ is seeded early from the VZ (i.e., prior to *GFAP-cre* expression) but does not require continual supply of progenitors from the VZ, as is the case for the cortical SVZ. There is, however, a contribution to the postnatal SVZ from ventral telencephalic radial glia, but this does not seem to occur until perinatal periods (Merkle et al., 2004).

Not all neurons generated at late stages of cortical development derive from SVZ progenitors. Some neurons are generated by asymmetric division of later-stage VZ progenitors (Noctor et al., 2004; Haubensak et al., 2004). Interestingly, however, these VZ-derived neurons spend a considerable time in the SVZ region (about 24 hr) before migrating out to the cortical plate (Noctor et al., 2004; Figure 1). This may serve to provide these newborn neurons with signals, which could specify their upper layer identity despite their VZ origin. From where or what would these putative signals derive? The ventrally derived GABAergic interneurons represent a significant population of cells in the cortical SVZ. These cells even share some of the molecular characteristics of the cortically derived SVZ progenitors (e.g., *Cux2* expression, see Zimmer et al., 2004). It is interesting to note that GABA has previously been shown to regulate proliferation in the cortical VZ and SVZ (Haydar et al., 2000). It is conceivable that GABA (or another factor from these cells) may also regulate the specification/differentiation of the late-born cortical neurons (see Figure 1). It would be interesting to know if mouse mutants (e.g., *Dlx1/2* or *Nkx2.1* mutants) that have reduced ventrally derived interneurons exhibit alterations in upper layer neuron formation.

It is likely that VZ-derived SVZ progenitors are intrinsically specified toward upper layer fates by virtue of their unique expression of the transcriptional regulators *Satb2* and *Cux2* or possibly the function of the gene *Svet1*. Moreover, these cells could, themselves, provide extrinsic influences to the VZ-derived neurons to initiate upper layer developmental programs. Therefore, the SVZ may serve as a specification station where late-born projection neurons are instructed to form the upper cortical layers. Hence, it is possible that, late stage, VZ progenitors remain multipotent and that signals/factors from the SVZ instruct the ultimate fate of their neuronal progeny. Indeed, the heterochronic transplantation studies mentioned earlier (Frantz and McConnell, 1996; Desai and McConnell, 2000) would likely have included a mixture of both VZ and SVZ progenitors. It would be interesting to perform these

studies with separately isolated (e.g., FACS-sorted) VZ and SVZ progenitors from the late-stage cortical germinal zone.

In summary, cortical neurogenesis is a temporally regulated process by which the progenitors become progressively restricted via both intrinsic and extrinsic mechanisms. In addition to timing of cell cycle exit, the location in the germinal zone (i.e., VZ versus SVZ) is likely to play an important role in cortical neuron specification.

Acknowledgments

I thank Gord Fishell, the anonymous reviewer, and members of my lab for their helpful comments and suggestions.

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